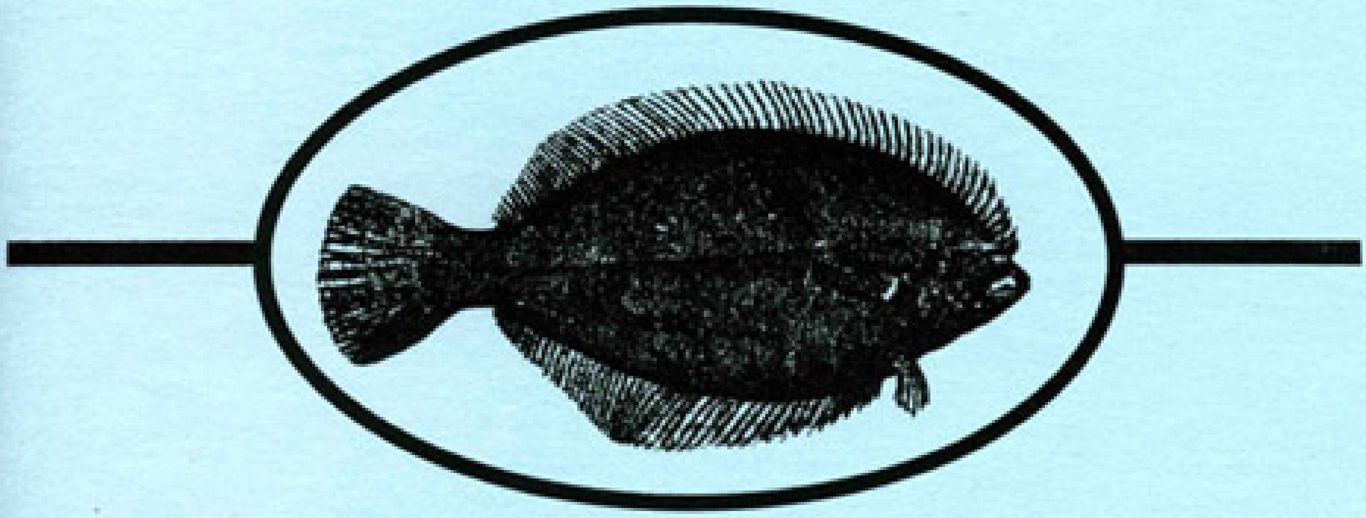
Program and Abstracts

Flatfish Biology Workshop

December 3-4, 1996
Mystic, Connecticut



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Flatfish Biology Workshop
December 3-4, 1996, Mystic, Connecticut

by Conference Steering Committee: Anthony Calabrese (Chair)[1],
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Fifth in a series of Flatfish Biology Conferences

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, Massachusetts

February 2008

Sponsored by

National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Flatfish Biology Workshop

December 3-4, 1996, Best Western Sovereign Hotel, Mystic, Connecticut

Oral Presentations

Tuesday, December 3rd

8:00 a.m. **Registration/Coffee-Best Western**

8:45 a.m. Welcome and Introduction
Anthony Calabrese, Conference Chair
National Marine Fisheries Service
Northeast Fisheries Science Center
Milford, CT

Michael Sissenwine, Directer
National Marine Fisheries Service
Northeast Fisheries Science Center
Woods Hole, MA

Session I

Penny Howell, Chair
Connecticut Department of Environmental Protection
Old Lyme, CT

9:00 a.m. Abundance and Distribution of Young-of-the-Year Winter Flounder (*Pleuronectes americanus*) in Point Judith Pond, RI
M. P. O'Connor
University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, Kingston, RI

9:20 a.m. Diel and Tidal Patterns of Distribution of Young-of-the-Year Windowpane Flounder, *Scophthalmus aquosus*
M. J. Newman and K. W. Able
Rutgers University Marine Field Station, Institute of Marine and Coastal Sciences, Tuckerton, NJ

9:40 a.m. Distribution and Abundance of Hogchoker, *Trinectes maculatus*, in Delaware Bay: Preliminary Comparisons between Restored and Reference Marshes
R. O. Bush and K. W. Able
Rutgers University Marine Field Station, Institute of Marine and Coastal Sciences, Tuckerton, NJ

10:00 a.m. Spatial Distribution of Juvenile and Adult American Plaice, *Hippoglossoides platessoides*, in the Georges Bank-Gulf of Maine Region
L. O'Brien
National Marine Fisheries Service, Woods Hole Laboratory, Woods Hole, MA

10:20 a.m. **Coffee Break**

Session II

Chris Powell, Chair
Rhode Island Division of Fish and Wildlife
Kingston, RI

10:40 a.m. Determinants of Depth Selection and Geographic Distribution of American Plaice in the Southern Gulf of St. Lawrence
D. P. Swain and R. Morin
Gulf Fisheries Centre, Department of Fisheries and Oceans, Moncton, New Brunswick, Canada

11:00 a.m. The Role of Estuarine Circulation Patterns in Regulating the Settlement of Juvenile Winter Flounder (*Pleuronectes americanus*) in Coves Near Ocean Inlets
M. C. Curran[1], R. J. Chant[2], K. W. Able[1], and S. M. Glenn[2]
[1]Rutgers University Marine Field Station, Tuckerton, NJ and [2]Institute of Marine and Coastal Sciences, New Brunswick, NJ

11:20 a.m. *Symphurus civitatium* Ginsburg, 1951 (Pleuronectiformes: Cynoglossidae) A Second Estuarine-dependent Tonguefish in Coastal Waters of the Southern United States
T. A. Monroe[1], R. L. Allen[2], D. M. Baltz[3], and S. Ross[4]
[1]National Marine Fisheries Service, National Systematics Laboratory, Washington, DC, [2]Louisiana State University, Department of Oceanography and Coastal Sciences, Baton Rouge, LA, [3]Louisiana State University, Coastal Fisheries Institute, Baton Rouge, LA, and [4]North Carolina National Estuarine Research Reserve, Wilmington, NC

11:40 a.m. Systematics and Distribution of Commercially Important Paralichthyid Flounders in Argentinean-Uruguayan Waters (*Paralichthys, Paralichthyidae*)
J. M. Diaz de Astarloa[1] and T. A. Munroe[2]
[1]Departmento de Ciencias Marinas, Facultad de Ciencias Exactas y Naturales Universidad Nacional de Mar del Plata, Argentina and [2]National Marine Fisheries Service, National Systematics Laboratory, Washington, DC

12:00 p.m. **Hosted Lunch**

Session III

Ambrose Jearld, Chair
National Marine Fisheries Service
Woods Hole, MA

1:00 p.m. Ciliated Epithelium in the Developing Digestive Tract of the Larva of the Atlantic Halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758)
C. Morrison
Department of Fisheries and Oceans, Aquaculture Division, Halifax, Nova Scotia, Canada

1:20 p.m. Cytochrome P450 Activity in Livers of Adult Winter Flounder from Boston Harbor and Cape Cod Bay
K. L. Wall[1], K. L. Jessen-Eller[2], and J. Crivello[1]
[1]University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT and [2]University of Connecticut, Department of Marine Sciences, Groton, CT

1:40 p.m. Light Microscopy and Mucus Histochemistry Study of the Developing Digestive Tract in Larval Yellowtail Flounder, *Pleuronectes ferruginea*, from 3-days Post-hatch to Metamorphosis
C. J. Baglole[1, 2], H. M. Murray[3], G. P. Goff[1, 2], and G. M. Wright[1]
[1]University of Prince Edward Island, Atlantic Veterinary College, Department of Anatomy and Physiology, Charlestown, PEI, Canada, [2]Huntsman Marine Sciences Centre, Department of Aquaculture, New Brunswick, Canada, and [3]Memorial University of Newfoundland, Ocean Sciences Centre, St. John's, Newfoundland, Canada

2:00 p.m. Control of Renal Excretion of Anionic Xenobiotics in Winter Flounder, *Pleuronectes americanus*
P. A. Halpin and J. L. Renfro
University of Connecticut, Department of Physiology and Neurobiology, Storrs, CT

2:20 p.m. Thyroid Hormone-salinity Interactions and Their Influences on Metamorphosis and Growth in Larval Summer Flounder
A. M. Schreiber[1], M. McArdle[1], B. Soffientino[1], A. Scribner[1], D. Bengtson[1], and J. Specker[2]
[1]University of Rhode Island, Department of Biological Sciences, Kingston, RI and [2]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI

2:40 p.m. **Cider and Cheese Break**

Session IV

Anne Studholme, Chair
National Marine Fisheries Service
Highlands, NJ

3:00 p.m. Relationships Between Life History Pattern and Recruitment in Flatfishes
C. Chambers
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

3:20 p.m. Nursery Habitat Preferences of Juvenile Flatfish on the Continental Shelf of the New York Bight
B. P. Stevens and R. K. Cowen
Marine Sciences Research Center, SUNY at Stony Brook, Stony Brook, NY

3:40 p.m. Midsummer Habitat Correlates and Patterns of Winter Flounder Distribution in the Navesink River and Sandy Hook Bay, New Jersey
J. P. Manderson, L. Stehlik, A. Stoner, J. Vitaliano, A. Bejda, F. Morello, J. Finn, B. Phelan, and S. Fromm
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

4:00 p.m. Habitat Use and Growth Patterns of Young-of-the-Year Winter Flounder in Three Northeastern U.S. Estuaries
B. A. Phelan[1], R. Goldberg[2], J. Pereira[2], P. Clark[2], A. J. Bejda[1], J. T. Finn[1], and S. A. Fromm[1]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]National Marine Fisheries Service, Milford Laboratory, Milford, CT

4:40 p.m. Trophic Linkages Between Different Estuarine Habitats and YOY Winter Flounder, as Determined Using Stable Isotope Ratios
S. C. Wainright[1], S. Y. Litvin[1], K. W. Able[1], A. L. Studholme[2], and A. Calabrese[3]
[1]Rutgers University, Institute of Marine and Coastal Sciences, New Brunswick, NJ, [2]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [3]National Marine Fisheries Service, Milford Laboratory, Milford, CT

5:00 p.m. **Poster Set-up**

5:30 p.m. **Hosted Mixer and Poster Session**

Wednesday, December 4th

8:00 a.m. **Registration/Coffee**

Session V

Don Danila, Chair
Northeast Utilities Environmental Laboratory
Waterford, CT

8:30 a.m. A Review of Milford Laboratory Studies on Effects of Contaminated Environments on Reproductive Success of the Winter Flounder, *Pleuronectes americanus*
F. P. Thurberg, D. A. Nelson, and J. J. Pereira
National Marine Fisheries Service, Milford Laboratory, Milford, CT

8:50 a.m. Reproductive Biology of Blackcheek Tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay, Virginia
M. R. Terwilliger
Virginia Institute of Marine Science, College of William and Mary, School of Marine Science, Gloucester Point, VA

9:10 a.m. Annular Growth Patterns, Age, and Longevity in a North Temperate Estuarine Species of Cynoglossid Tonguefish, *Symphurus plagiusa*, with Comparisons of Growth Parameters among Sympatric Pleuronectiformes
M. R. Terwilliger
Virginia Institute of Marine Science, College of William and Mary, School of Marine Science, Gloucester Point, VA

9:30 a.m. Growth and Mortality of Juvenile Winter Flounder in Two New England Estuaries
C, Meise[1], J. S. Collie[2], J. Widman[3], and P. Howell[4]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, [2]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI,[3] National Marine Fisheries Service, Milford Laboratory, Milford, CT, and [4]State of Connecticut Department of Environmental Protection, Marine Headquarters, Old Lyme, CT

9:50 a.m. Associations Between Liver Lesions in Winter Flounder (*Pleuronectes americanus*) and Sediment Chemical Contaminants from Northeast United States Estuaries
S. Chang[1], V. S. Zdanowicz[1], and R. A. Murchelano[2]
[1]National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ, and [2]National Marine Fisheries Service, Woods Hole Laboratory, Woods Hole, MA

10:10 a.m. **Coffee Break**

Session VI

Allan Beck Chair

Environmental Advantage Group
Prudence Island, RI

10:30 a.m. Affects of Larval Diets and Light Intensity on Growth, Survival and Pigmentation of Southern Flounder
M. R. Denson and T. J. Smith
South Carolina Department of Natural Resources, Charleston, SC

10:50 a.m. Current Status of Atlantic Halibut Culture in the Maritimes
K. G. Waiwood
Department of Fisheries and Oceans, St. Andrews Biological Station, St. Andrews, New Brunswick, Canada

11:10 a.m. Reproduction of American Plaice, *Hippoglossiodes platessoides*, and Turbot, *Reinhardtius hippoglossiodes*, in Newfoundland Waters
D. M. Maddock, R. M. Rideout, and M. P. M. Burton
Memorial University of Newfoundland, Department of Biology and Ocean Sciences Centre, St. John's, Newfoundland, Canada

11:30 a.m. Investigations into the Causes of Early Larval Mortality in Cultured Summer Flounder
D. Alves[1], D. A. Bengtson[2], and J. L. Specker[3]
[1]University of Rhode Island, Department of Biological Science, [2]University of Rhode Island, Department of Fisheries, Animal and Veterinary Science, and [3]University of Rhode Island, Graduate School of Oceanography, Kingston, RI

11:50 a.m. Progress in Controlled Maturation and Spawning of Summer Flounder (*Paralichthys dentatus*) Broodstock
W. O. Watanabe[1], E. P. Ellis[1], S. C. Ellis[1], M. W. Feeley[2], and R. A. Cooper[2]
[1]Caribbean Marine Research Center, Vero Beach, FL, and [2]University of Connecticut, Marine Sciences and Technology Center, Groton, CT

12:10 p.m. Production of Summer Flounder at Great Bay Aquafarms
G. Nardi
Great Bay Aquafarms, Portsmouth, NH

12:30 p.m. **Hosted Lunch**

Adjourn

Poster Session

Tuesday, December 3rd, 5:30 p.m.

Can Scales be Used to Sex Winter Flounder (*Pleuronectes americanus*)
A. J. Bejda and B. A. Phelan
National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ

Maturity Stages of American Plaice in the Gulf of St. Lawrence
K. Benhalima and R. Morin
Department of Fisheries and Oceans, Gulf Fisheries Centre, Moncton, New Brunswick, Canada

Witch Flounder (*Glyptocephalus cynoglossus*) Aquaculture
D. Bidwell, W. H. Howell, N. King, E. Fairchild, and A. Tomlinson
University of New Hampshire, Coastal Marine Laboratory, Newcastle, NH

Pleurocidin-An Antimicrobial Peptide from the Skin Secretions of Winter Flounder
A. M. Cole, P. Weis, and G. Diamond
University of Medicine and Dentistry of New Jersey, Department of Anatomy, Cell Biology, and Injury Sciences, Newark, NJ

Effect of Density on the Growth of Summer Flounder, *Paralichthys dentatus*
E. Fairchild, W. H. Howell, A. Tomlinson, and N. King
University of New Hampshire, Coastal Marine Laboratory, Newcastle, NH

Cloning and Sequencing of Winter Flounder Digestive Enzyme Genes
J. W. Gallant and S. E. Douglas
Institute for Marine Biosciences, Halifax, Nova Scotia, Canada

Juvenile Winter Flounder Injected Subcutaneously with Elastomer: Movements and Mortality Estimates in Two Selected Coastal Salt Ponds
C. L. Gray and M. R. Gibson
Rhode Island Fish and Wildlife, Marine Sciences Section, Wickford, RI

Rearing Juvenile Summer Flounder, *Paralichthys dentatus,* in Different Temperature and Salinity Combinations to Enhance Growth for Aquaculture
G. Klein-MacPhee[1] and M. O'Connor[2]
[1]University of Rhode Island, Graduate School of Oceanography, Narragansett, RI, and
[2]University of Rhode Island, Department of Resource Development, College of Fisheries, Aquaculture and Veterinary Science, Kingston, RI

Ecology of Sheepscot Bay Winter Flounder
R. Langton, S. Sherman, C. Simard, B. Joule, H. Perkins, and S. Chenoweth
Maine Department of Marine Resources, West Boothbay Harbor, ME

Light Intensity and Salinity Effects on Eggs and Yolk-sac Larvae of the Summer Flounder, *Paralichthys dentatus*
W. O. Watanabe[1], M. W. Feeley[2], S. C. Ellis[1], E. P. Ellis[1], and R. A. Cooper[2]
[1]Caribbean Marine Research Center, Vero Beach, FL and [2]University of Connecticut, Marine Science and Technology Center, Groton, CT

Abstracts

Oral Presentations

Abundance and Distribution of Young-of-the-Year Winter Flounder (*Pleuronectes americanus*) in Point Judith Pond, Rhode Island

M. P. O'Connor

University of Rhode Island
Department of Fisheries, Animal and Veterinary Science
Kingston, RI 02880

Abundance and distribution of young-of-the-year winter flounder was determined July through October 1996 in Point Judith Pond, RI. The flounder were captured using an enclosed quadrat and then marked subcutaneously with Alcian blue dye with the Panjet inoculator. Recaptures occurred throughout the period and were marked again. The reason for this mark recapture study is to observe young-of-the-year winter flounder movements and habitat requirements. Habitat will be described primarily by the distinctive flora and grain size found in the various sites. Abundance will be compared among the different habitats described. Final results will be discussed.

Diel and Tidal Patterns of Distribution of Young-of-the-Year Windowpane Flounder, *Scopthalmus aquosus*

M. J. Neuman and K. W. Able

Rutgers University Marine Field Station
Institute of Marine and Coastal Sciences
800 Great Bay Boulevard
Tuckerton, NJ 08087

We investigated the diel and tidal patterns of estuarine habitat use by young-of-the-year (YOY) windowpane flounder, Scophthalmus aquosus, at a dynamic sandy beach island just inside Little Egg Inlet, New Jersey. We sampled during each tidal stage (mid-ebb, low, mid-flood, high), over a 24-hr period, in July and August 1994 and 1995 at two sites (depth $\leq$ 1.4 m at mean low water). Windowpane (n=456, 14.0-148.0 mm TL) were collected with a 6.1-m bag seine (3-mm mesh, 192 hauls) and a 1.0-m beam trawl (3-mm mesh, 192 tows). For both years: 1) windowpane abundance was higher in July than in August (1.86 and 0.51 number/tow, respectively); 2) individuals collected in July were smaller than those collected in August (14.0-143.0 and 40.0-148.0 mm TL, respectively); 3) abundance was higher for the seine than for the beam trawl (2.12 and 0.26 number/tow, respectively); and 4) abundance was higher at the site on the west side of the island than at the site on the southwest side of the island (2.15 and 0.22 number/tow, respectively). Despite these differences between gears, months and sampling sites, windowpane abundances were typically higher during low tides than at other tidal stages. Time of day (dawn, day, dusk, night) had no significant effect on abundance. However, in all cases the lowest catches were recorded during the day.

These data provide evidence that YOY windowpane use inlet beaches as nurseries. Among inlet beach habitats, windowpane abundances may vary due to differences in substrate type, flow rate and/or abundance of prey. We believe that the higher catches observed during low tides may be because: 1) windowpane are moving onto the beach where they are vulnerable to our gears; or 2) only at low tide can our sampling cover areas where windowpane are present.

Distribution and Abundance of Hogchoker, *Trinectes maculatus*, in Delaware Bay: Preliminary Comparisons between Restored and Reference Marshes

R. O. Bush and K. W. Able

Rutgers University Marine Field Station
Institute of Marine and Coastal Sciences
800 Great Bay Boulevard
Tuckerton, NJ 08087

As part of an intensive study of marshes in Delaware Bay, we are evaluating the effectiveness of marsh restoration by examining juvenile fish distribution and abundance in a variety of restored and reference marshes. In preliminary studies during 1996 we sampled in intertidal and subtidal habitats with weirs (n=54) and otter trawls (n=561), respectively. Over the salinity range sampled (0-16 ppt), Trinectes maculatus appear most abundant in creeks at intermediate salinities where they were dominated by 50-80 mm TL (range 25-170) individuals that are approximately 1-2 years old, based on a study conducted by Mansueti and Pauly (1956). Curiously, YOY are absent from our samples to date. In one reference creek system (Madhorse Creek, 10-12 ppt range) in which hogchoker were a dominent component of the fish fauna, the distribution varied with habitat type and season. They seldom occurred on the intertidal marsh surface, were slightly more abundant in shallow subtidal creeks <1m MLW, and most abundant in deeper 2-3 m subtidal creeks. Further studies will continue to evaluate the patterns of habitat use with emphasis on YOY.

Spatial Distribution of Juvenile and Adult American Plaice, *Hippoglossoides platessoides*, in the Georges Bank-Gulf of Maine Region

L. O'Brien

National Marine Fisheries Service
Woods Hole Laboratory
166 Water Street, Woods Hole, MA 02543

The influence of depth, bottom temperature, and sediment type on the distribution of American plaice in the Georges Bank-Gulf of Maine region was explored using a generalized additive model (GAM). Length data collected on Northeast Fisheries Science Center autumn bottom trawl surveys during 1963-1995 were disaggregated into inshore and offshore components and four length groups: immature (1-15 cm); primarily immature (16-23 cm); primarily mature (24-34 cm); and fully mature (> 35 cm) fish. A GAM was fit to each length group to detect any changes in preferred habitat with changes in size. Results indicate that juveniles, in general, occupy shallower depths than adults but temperature does not appear to influence this distribution.

Determinants of Depth Selection and Geographic Distribution of American Plaice in the Southern Gulf of St. Lawrence

D. P. Swain and R. Morin

Gulf Fisheries Centre
Department of Fisheries and Oceans
Moncton, New Brunswick, Canada EOG 2XO

American plaice, a dominant groundfish species in the southern Gulf of St. Lawrence, has varied five fold in abundance since 1971. The stock has occupied the same geographic range in periods of high and low abundance, maintaining highest abundance in the same sectors of the Gulf during both periods. The area of highest plaice density (preferred areas) tended to expand with population size, but similar expansion was not observed into marginal areas less frequented by plaice. These results are contrary to predictions based on optimal foraging theory and contrast with the pattern observed for Atlantic cod in the southern Gulf. Plaice density was strongly related to depth, with peak densities at intermediate depths of 70-90 m. Contrary to the widespread tendency for juvenile fishes to occupy shallower depths, plaice depth selection was similar for all ages (3-12+ yr) and varied little over time or with population abundance. Plaice density was maximum at the same depths for males and females, but the depth distribution of females tended to be more spread out than males. Females also had a broader geographic range than males of the same age or size. The broad geographic range and depth selection of female plaice, relative to males, are consistent with higher growth rates for females and may reflect a higher foraging rate and more competitive interactions.

The Role of Estuarine Circulation Patterns in Regulating the Settlement of Juvenile Winter Flounder (*Pleuronectes americanus*) in Coves Near Ocean Inlets

M. C. Curran[1], R. J. Chant[2], K. W. Able[1], and S. M. Glenn[2]

[1]Rutgers University Marine Field Station
Institute of Marine and Coastal Sciences
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Tuckerton, NJ 08087

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Institute of Marine and Coastal Sciences
PO Box 231
New Brunswick, NJ 08903-0231

Prior research has indicated that coves in the vicinity of Little Egg Inlet in southern New Jersey are settlement areas for the winter flounder, *Pleuronectes americanus*. The goal of the present research was to determine whether the spring circulation pattern in Little Egg Harbor supports the advection of larvae into these coves. The survey consisted of repeated transects using an Acoustic Doppler Current Profiler (ADCP) over a 12-hr tidal cycle. Additionally, two S4 current meters were placed in the harbor near the mouth of one cove over a three-week period during flounder settlement (April-May). Our results indicated that there is a strong northward flow of water from the inlet into the western side of the harbor during the flooding current, and, conversely, there is southward flow of water on the eastern side of the harbor during the ebbing current. Therefore, larvae that hatch in the northern portion of Little Egg Harbor may travel down the eastern side of the harbor during ebbing current and be advected into the coves. Based on plankton tows, more winter flounder larvae were found entering the cove on the flooding current than leaving on the ebbing current, supporting the conclusion that they are moving into the cove. Furthermore, the timing of the decline in larval abundance corresponded to the increase in recently settled benthic juveniles collected by beam trawls. The maximum densities of these juveniles occurred in the cove and this number far exceeded values from outside the cove. Our results have important implications for the population dynamics of winter flounder.

Symphurus civitatium Ginsburg, 1951 (Pleuronectiformes: Cynoglossidae) A Second Estuarine-dependent Tonguefish in Coastal Waters of the Southern United States

T. A. Monroe[1], R. L. Allen[2], D. M. Baltz[3], and S. Ross[4]

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National Systematics Laboratory
MRC-153 National Museum of Natural History
Washington, DC 20560

[2]Louisiana State University, Department of Oceanography and Coastal Sciences
Baton Rouge, LA 70803-7503

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Baton Rouge, LA 70803-7503

[4]North Carolina National Estuarine Research Reserve
7205 Wrightsville Avenue
Wilmington, NC 28403

Two tonguefish species, Symphurus plagiusa (Linnaeus) and S. civitatium Ginsburg occur sympatrically in coastal shallow-water habitats in the southeastern United States (including North Carolina and Louisiana estuaries). Approximately 2% of 400 juvenile tonguefishes from North Carolina estuaries were S. civitatium, while in Barataria Bay, LA, this species was very abundant and cosmopolitan in distribution comprising nearly 65% of 3600+ tonguefishes collected with a small beam trawl. Historical literature has regarded S. plagiusa as the only tonguefish species inhabiting inshore habitats in these regions. Presence of a second Symphurus species in estuarine regions has been overlooked most probably due to difficulties in identifying post-settlement and early juvenile stages of these species. Early life history stages of both species, although similar in morphology and with overlapping finray counts, can readily be recognized by differences in caudal-fin rays (10 in S. plaguisa and 12 in S. civitatium), especially when used in combination with other characters including pigmentation patterns, jaw position relative to lower eye position, and morphometric features. Occurrence of two sympatric species of tonguefishes in estuarine environments in the central Gulf of Mexico and southeastern regions of the U.S. potentially compromises results of earlier ecological and distributional studies that assumed presence of only a single tonguefish species in these habitats.

Systematics and Distribution of Commercially Important Paralichthyid Flounders in Argentinean-Uruguayan Waters (*Paralichthys, Paralichthyidae*)

J. M. Diaz de Astarloa[1] and T. A. Monroe[2]

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Species of *Paralichthys* Girard, 1858 are the most valuable flatfishes in demersal fisheries of Argentinean and Uruguayan waters. Recent commercial catches increased from 3,000 T in 1984 up to 11,000 T in 1995, representing more than $65 million in export income for 1995. Although their commercial importance has long been recognized, paralichthyid flounders in this region are not well studied either from a systematic or ecological view point. Six nominal species are reported from Argentinean and Uruguayan waters: *P. bicyclophorus* Ribeiro, 1915, *P. brasiliensis* Ranzani, 1840, *P. isosceles* Jordan, 1890, *P. orbignyanus* Valenciennes, 1839, *P. patagonicus*, Jordan, in Jordan & Goss, 1889, and *P. simulans* Lahille, 1939. However, species descriptions are often vague and incomplete, and reported distributions are dubious. *Paralichthys* sp. were collected on the continental shelf and in estuaries in the south Atlantic between 34-55ºS. Variations in scale morphology, meristic and morphometric characters, osteology and coloration were used to differentiate among *Paralichthys* species co-occurring in this area. Of six species previously reported from this region, only three, *P. isosceles, P. orbignyanus* and *P. patagonicus*, are valid. *Paralichthys bicycloporus* and *P. simulans* are junior synonyms; while *P. brasiliensis*, although reported from coastal waters of Argentina and Uruguay for many years, does not occur here. *Paralichthys orbignyanus* and *P*. *patagonicus* inhabit marine and estuarine locations and extend as far south as 43ºS. The former is a shallow-water euryhaline species occurring to about 20 m. In contrast, *P*. *patagonicus* has higher densities between 34-40 ºS at 80-100 m depth. *Paralichthys isosceles* occurs mainly on the inner continental shelf between 43-45 ºS at 70-100 m depth, and reaches its southern limit at about 47 ºS.

Ciliated Epithelium in the Developing Digestive Tract of the Larva of the Atlantic Halibut, *Hippoglossus hippoglossus* (Linnaeus, 1758)

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There are two regions of ciliated epithelium in the digestive tract of the halibut larva. The anterior region, present by 5 days post-hatch and still present at 25 days post-hatch, probably moves water through the brachial openings into the oesophagus and on to the midgut until the mouth forms at about 29 days post-hatch. A ciliated region is also present in the occluded posterior hindgut, which joins the excretory duct to form a common duct to the exterior. This posterior ciliated region may help to circulate water in the mid- and hindgut until the anus opens directly to the exterior, at about 25 days post-hatch.

Cytochrome P450 Activity in Livers of Adult Winter Flounder from Boston Harbor and Cape Cod Bay

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In conjunction with the New England Aquarium-sponsored Fish Day, in May of 1995 local fishermen collected adult winter flounder from various sites within Boston Harbor. During a separate collection effort the National Marine Fisheries Service collected adult winter flounder from Cape Cod Bay for use as reference samples. Samples of the liver from each individual were excised and a variety of parameters measured. For all fish sampled cytochrome P450 IA1 activity, as measured by Ethoxyresorufin O-dethylase (EROD), was significantly higher in females than males.

At the temperature optimum for the assay (18ºC) females from Cape Cod Bay had significantly higher EROD activity than Boston Harbor females. However, this difference was not apparent when samples were assayed at the ambient temperature of the water from which fish were captured. Cytochrome P450 2E1-like activity, as measured by Chloroxazone 6-hydroxylation, exhibited an opposite trend, such that females had a significantly lower activity than males. Also, at presumed ambient temperatures females from Cape Cod Bay had lower activities than Boston Harbor females, where as no difference was observed at the temperature optimum. Interestingly, it was observed that % liver lipid and total liver protein were significantly lower in Boston Harbor vs. Cape Cod females. However, no direct correlation could be made between these parameters and either of the P450 activities measured. Although P450 IA1 activity has been correlated to exposure to polycyclic aromatic hydrocarbons and polychlorinated biphenyls in a variety of marine species, these results suggest that enzyme activity alone may not serve as a reliable indicator. Also, this work demonstrates the importance of reporting ambient and assay temperature. In mammals, P450 2E1-like activity has been correlated with bioactivation of a variety of small volatile organic compounds such as acetone, trichloroethylene, and vinyl chloride. Thus, it is noteworthy that activity of this compound could be detected at all in feral fish populations.

At this point no conclusions can be made regarding presence of inducting agents of this enzyme activity. As in mammals, physiological factors such as starvation also play a role in P450 2E1 regulation.

Light Microscopy and Muscus Histochemistry Study of the Developing Digestive Tract in Larval Yellowtail Flounder, *Pleuronectes ferruginea*, from 3-Days Post-hatch to Metamorphosis

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Successful feeding of larval yellowtail flounder, Pleuronectes ferruginea, is an essential prerequisite to the aquaculture development of this species. This study examines the major ontogenetic changes occurring in the digestive tract of larval yellowtail flounder between 3 and 46 days following hatch using light microscopy and mucus histochemistry.

The digestive tube of larvae fixed in toto in Bouin's fluid was undifferentiated at 3-days post-hatch. Regional differentiation was observed by 7-days post-hatch and by day 10 was complete. Six distinct regions were defined: the buccal cavity, pharynx, esophagus, post-esophageal swelling (PES), intestine and rectum. Goblet cells were present in the buccal cavity, pharynx, and intestine by day 7. A further distinction by day 29 between zone 1 (anterior) and zone 2 (posterior) of the esophagus was based on an increase in goblet cell numbers in zone 2. The PES was defined as a stomach with the appearance of multicellular glands by day 36. The liver was present by day 3 and the pancreas between days 3 and 7. Goblet cells and the apical layer of stratified epithelia lining the buccal cavity, pharynx, and esophagus were strongly positive for acid mucins. The epithelia of the PES/stomach stained weakly for neutral mucins. No mucin staining was associated with the gastric glandular epithelium. The brush border of the intestine and rectum stained strongly for a combination of neutral and acid mucins.

Future work includes lectin-binding studies to determine structural differentiation and enzyme histochemistry to evaluate digestive enzyme activity and distribution.

Control of Renal Excretion of Anionic Xenobiotics in Winter Flounder, *Pleuronectes americanus**

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The rate of excretion of toxic pollutants such as the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D) can determine the dwell time within the body and thus potential toxicity. Factors which increase or decrease the rate of pollutant excretion may impact significantly the animal's health. Prior studies had shown that monolayer cultures of winter flounder renal proximal tubule cells (PTCs) were capable of active transepithelial transport of 2, 4-D. In addition, factors such as phorbol esters, which stimulate protein kinase C (PKC), inhibited 2, 4-D secretion, indicating that intracellular signaling systems could modulate secretion. The renal clearance of organic anions is known to vary *in vivo.*

To assess possible regulatory control, we mounted PTCs in Ussing chambers and examined the unidirectional fluxes of ^{14}C-2, 4-D. Test of potential primary messengers of the PKC effect showed that 1 µM dopamine, from the peritubular side only, significantly ($P<0.05$) inhibited net 2, 4-D secretion (control: 1.03 ± 0.07; dopamine: 0.89 ± 0.07). Others have shown that dopamine partially deactivates Na,K-ATPase and –adrenergic agonists stimulate Na,K-ATPase activity in proximal tubule. In PTCs, we found the –adrenergic agonist, oxymetazoline (10µM), transiently, but significantly ($P<0.05$), stimulated 2, 4-D secretion (142% of control).

To examine involvement of the sodium gradient, tissues were preincubated 1 hr in 10 µM 2, 4-D with Na^+-free medium on the luminal side only. After 1 hr, Na^+ was restored to the lumen. Both Na-dependent glucose transport and 2, 4-D secretion were stimulated by this transient increase in the plasma membrane Na^+ gradient. The data indicate that catecholamine may greatly influence rates of pollutant excretion in flounder.

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Thyroid Hormone-salinity Interactions and Their Influences on Metamorphosis and Growth in Larval Summer Flounder

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Summer flounder metamorphosis is characterized in part by the migration of the right eye to the left side of the head, a process which permits classification of different larval stages. Thyroid hormones (THs) are believed to directly mediate metamorphosis in all flatfish species, though this hypothesis has only been confirmed for the Japanese flounder. In this study we show that 1) THs are both necessary and sufficient for metamorphosis in summer flounder, 2) a natural TH surge during metamorphic climax is accompanied by an increased growth rate; the chemical inhibition of this surge inhibits growth, though TH supplementation does not enhance growth, 3) larval osmoregulatory tolerances fluctuate during the progression of metamorphosis, 4) salinity interacts with the thyroid axis in a stage-dependent manner, and 5) salinity influences development and growth. The effect of salinity on development and growth may be mediated through an interaction with the thyroid axis.

Relationships Between Life History Pattern and Recruitment in Flatfishes

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Flatfishes are one group for which data on life history and population characteristics are fairly extensive. I have evaluated data from the literature and other historical sources for the purpose of establishing relationships between life history traits and various measures of recruitment and stock dynamics of flatfishes. Variables of particular interest included features of the early life history (*e.g.*, sizes of eggs and larvae, sizes at settlement or metamorphosis, larval period duration) and those depicting variability in yearclass strength (*e.g.*, CV of abundance of yearclasses). The patterns revealed by the analysis and their implications will be presented, as will suggestions for further work. Among other benefits, this kind of exploratory analysis has utility for establishing a broader context from which species-level investigations on recruitment processes can proceed.

Nursery Habitat Preferences of Juvenile Flatfish on the Continental Shelf of the New York Bight

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Nearshore environments such as estuaries have long been given particular emphasis in the study of fish nursery habitats. Some fish species, however, utilize offshore regions such as continental shelves as their nursery habitat, yet little work has been directed at their habitat requirements. To examine potential offshore nursery areas for juvenile flatfish, cruises in the New York Bight were conducted monthly, between June 1996 to October 1996. A modified 2-m beam trawl was utilized across three transects of seven stations each ranging in depth from 20 to 100 meters. Abundances and distributions of several species including yellowtail flounder, *Limanda ferruginea*, and Gulf Stream flounder, *Citharichthys arctifrons*, indicate relatively discrete juvenile species assemblages across the continental shelf. Furthermore, changes in patterns of distribution for several species during the course of the study indicate possible immigration/emigration and differential mortality processes among sites. These data will be discussed with respect to nursery habitat preferences for offshore flatfish species of the New York Bight's continental shelf.

Midsummer Habitat Correlates and Patterns of Winter Flounder Distribution in the Navesink River and Sandy Hook Bay, New Jersey

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An exploratory survey of the Navesink River and Sandy Hook Bay, New Jersey was performed to begin to examine the influence of landscape pattern on estuarine community interactions. Survey collections were made in late July and early August with beam and otter trawls at 89 fixed stations. Stations were located in a variety of habitats throughout the estuary, including those considered important to winter flounder. A preliminary multivariate analysis was performed to identify the dominant species assemblages, species associations, and important ecological gradients in the system.

Winter flounder (N=196, 33-305 mm total length (TL)) ranked 4th in total abundance among fish species collected and over 90% of the individuals were young-of-the-year (33-117 mm TL). Young-of-the-year flounder were relatively abundant in a variety of shallow water habitats throughout the study area, including marsh creeks and marsh-fringed coves, sandbars, and in habitats characterized by dense aggregations of the amphipod *Ampelisca abdita*. The species frequently co-occurred with the blue crab *Callinectes sapidus* and fluke *Paralichthys dentatus*, which are potentially important predators.

Habitat Use and Growth Patterns of Young-of-the Year Winter Flounder in Three Northeastern U. S. Estuaries

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A comparative study was designed to assess the quality of selected nursery habitats for young-of-the-year (YOY) winter flounder (*Pleuronectes americanus*) in Connecticut and New Jersey. Habitat-specific abundance, distribution, and growth were quantified in common nursery habitats. Habitat types selected for study were characterized by unattached macroalgae (*Ulva lactuca*), eelgrass (*Zostera marina*), and adjacent unvegetated areas and marsh creeks. Habitat-use patterns were determined quantitatively using a 1-meter beam trawl. Young-of-the-year winter flounder utilize primary settlement areas and disperse from them to a variety of vegetated and unvegetated habitats. Eelgrass habitat and adjacent unvegetated areas supported a greater number of YOY than other habitats. Growth was quantified by measuring increases in length and weight of fish held in short-term caging experiments at specific habitat types. Survival and growth varied with habitat type. The greatest growth rates occurred for the smallest individuals and rapidly diminished with time. Comparisons among habitats indicated growth to be similar except for marsh creeks, which were lower. In salt marsh creeks where dissolved oxygen levels were often very low, survival was poor.

These data suggest that eelgrass, macroalgae, and adjacent unvegetated areas are important as nurseries for winter flounder expecially when environmental conditions (*i.e.*, dissolved oxygen and water temperatures) are favorable. Concurrent interpretive studies on stable isotope ratios, RNA/protein levels, and sediment electrochemistry, support conclusions drawn from caging experiments.

Trophic Linkages Between Estuarine Habitats and YOY Winter Flounder, as Determined Using Stable Isotope Ratios

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During the past three years, under the auspices of NOAA's Coastal Ocean Program, we have been conducting studies on habitat utilization and habitat-specific growth in juvenile winter flounder and tautog. These studies have been conducted in three northeastern estuaries (Great Bay/Little Egg Harbor, Long Island Sound, and the Hudson/Raritan), chosen so as to represent a gradient of anthropogenic influence. At a previous workshop, we presented preliminary evidence from the 1st year, showing that YOY winter flounder which were confined to cages within certain habitats *(e.g.,* eelgrass beds) may assume distinctive stable isotopic compositions. We confirmed this pattern with additional caging experiments conducted during the past 2 years. Similar experiments using juvenile tautog indicated that habitat-specific patterns exist for this species as well. In addition to the habitat-specific isotopic compositions within estuaries, we have seen a recurring pattern of higher nitrogen isotope values in estuaries with more human influence, *i.e*., the Hudson-Raritan and Long Island Sound estuaries. Taken together, our studies indicate that nitrogen isotope ratios may be an indicator of anthropogenic stress in aquatic ecosystems, and that carbon and nitrogen stable isotopes may be used to elucidate patterns of habitat utilization by at least some juvenile fishes within estuaries.

A Review of Milford Laboratory Studies on Effects of Contaminated Environments on Reproductive Success of the Winter Flounder, *Pleuronectes americanus*

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A series of studies on effects of contaminated habitats on reproductive success of the winter flounder have been conducted at the Milford Laboratory in recent years. Flounder collected from polluted urban harbors and from relatively uncontaminated coastal areas were spawned in the laboratory and the resulting eggs and larvae were evaluated for hatching success and larval development. Reduced hatch and impaired larval development were found in fish from contaminated urban harbors when the eggs and larvae were compared to those obtained from flounder taken from cleaner habitats.

Harbor dredging has been suspected as an impediment to flounder recruitment when dredging takes place during the spawning season. When dredging conditions were simulated in the laboratory, reduced hatching success was noted in winter flounder eggs. Both clean and contaminated dredge spoils were used in these simulations. Preliminary results show that although the greatest reduction in hatching success was found with contaminated sediments, both sediments cause some impairment indicating that factors other than chemical contamination may be involved.

The role of a contaminated habitat on the production of the egg-yolk precursor protein vitellogenin was also studied in winter flounder and the results obtained demonstrate contaminant-induced changes in vitellogenin production and transport in these fish.

Reproductive Biology of Blackcheek Tonguefish, *Symphurus plagiusa* (Cynoglossidae: Pleuronectiformes), in Chesapeake Bay, Virginia

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The blackcheek tonguefish**,** *Symphurus plagiusa,* is the only member of the pleuronectiform family Cynoglossidae occurring in Chesapeake Bay. From Chesapeake Bay and southward it is an abundant component of the fish fauna occurring in estuaries and coastal embayments. Despite its relative abundance in different estuarine and marine habitats of the northwest Atlantic, information on the life history parameters of the species is limited. The reproductive biology of *S. plagiusa* was studied of specimens collected via the Virginia Institute of Marine Science (VIMS) juvenile finfish and blue crab stock assessment programs trawl survey of the lower Chesapeake Bay and the three major Virginia tributaries: the York, James and Rappahannock Rivers. Among 556 specimens examined in this study, sex ratio was 223 males (36-190 mm TL) to 343 females (46-202 mm TL), or 1:1.54. Macroscopic inspection of gonadal tissue showed that this species exhibits a protracted spawning period from late May to September. Histological examination of ovarian tissue reinforces these findings. Size frequency distributions of oocytes show no hiatus between oocyte size classes, indicating the *S. plagiusa* is an indeterminate spawner. Based upon reproductive stages of females, it is estimated that *S. plagiusa* matures by age one. Batch and total fecundity estimates will be completed in the near future. The reproductive parameters of blackcheek tonguefish will be compared to those of five sympatric Pleuronectiformes.

Annular Growth Patterns, Age and Longevity in a North Temperate Estuarine Species of Cynoglossid Tonguefish, *Symphurus plagiusa*, with Comparisons of Growth Parameters among Sympatric Pleuronectiformes

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Blackcheek tonguefish, *Symphurus plagiusa*, is the only cynoglossid flatfish occurring in abundance in shallow estuaries and coastal embayments in north temperate latitudes. In Chesapeake Bay, it ranks sixth in abundance in juvenile finfish surveys. Transverse sections of 566 sagittal otoliths were read for ageing. Growth marks are formed once yearly in June. The first annulus is formed at a size range of 88-138 mm TL. Macroscopic staging of ovaries revealed that blackcheek tonguefish reached sexual maturity between 80-130 mm TL. Length at 50% maturity was 97 mm TL. Von Bertalanffy growth parameters were similar for both sexes; Von Bertalanffy equations were: males: $L_t = 196.5\ (1-e^{-.2853(t+.9195)})$; females: $L_t = 190.6\ (1-e^{-.3205(t+.7842)})$. Growth is rapid during the first year for both sexes, then slows rapidly thereafter. Maximum age for both sexes was 5 years. This study revealed overlap in total length between age groups, thus rendering age interpretations based on length frequency unreliable. Detailed growth studies for other cynoglossid species are unavailable; no comparisons could be made among other members of this family. Growth parameters of blackcheek tonguefish were compared to those of five sympatric Pleuronectiformes. A variety of growth schemes are exhibited by these Pleuronectiformes, indicating that several growth patterns exist in fish that successfully endure the rigors of dynamic estuarine environments.

Growth and Mortality of Juvenile Winter Flounder in Two New England Estuaries

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We compared young-of-the-year winter flounder (*Pleuronectes americanus*) populations from a contaminated site, New Haven Harbor, and a clean site, the Connecticut River estuary. Juvenile winter flounder were collected with a 1-m beam trawl at bi-monthly intervals during 1993. This analysis is limited to 3- to 15-cm winter flounder collected from June to October. We fit a length-based model to the observed size-frequency distributions. The mode incorporates the von Bertalanffy growth equation and a mortality rate that decreases exponentially with size. A bootstrap algorithm was used to generate confidence intervals for the growth and mortality coefficients. Growth and mortality rates were significantly higher at the contaminated site than at the clean site.

Associations Between Liver Lesions in Winter Flounder (*Pleuronectes americanus*) and Sediment Chemical Contaminants from Northeast United States Estuaries

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Neoplastic diseases, particularly of bottom-dwelling fishes, are more prevalent in coastal areas than in areas that are relatively pristine. Although sediments in many urbanized estuaries contain high concentrations of contaminants, there is no conclusive evidence linking a specific organic or inorganic chemical to a particular liver lesion in winter flounder (Pleuronectes americanus). Between 1984 and 1986, sediments and winter flounder were collected from 10 sites in the northeast United States ranging from grossly polluted to relatively unimpacted. Sediments were analyzed for polycyclic aromatic hydrocarbons (PAHs), chlorinated pesticides, polychlorinated biphenyls (PCBs), and metals. Gross and microscopic pathological examinations were conducted on winter flounder liver sections. Factor and canonical correlation analysis were used to explore associations between biological and chemical measurements.

In general, inflammatory liver lesions (hepatitis, cholangitis, phlebitis and macrophage aggregate hyperplasia) showed strong positive associations with low molecular weight, petroleum-derived PAHs, tri- to hexa-chlorobiphenyls and chromium, lead, thallium and selenium, but were negatively associated with DDT-type pesticides. Pre/neoplastic lesions (cytoplasmic hepatocellular vacuolation, cytoplasmic bile duct vacuolation and preneoplasia) showed strong positive associations with most PAHs measured, whether petroleum- or combustion-derived, the pesticides dieldrin, transnonachlor and alpha-chlordane, and silver, copper, antimony and tin, but no associations with PCBs were found. Necrotic lesions (necrosis, cell necrosis and hemorrhagic necrosis) showed strong positive associations with hepta- to nona-chlorobiphenyls and arsenic, zinc, nickel, and mercury, and strong negative associations with high molecular weight, combustion-derived PAHs and DDT metabolites.

Effects of Larval Diets and Light Intensity on Growth, Survival, and Pigmentation of Southern Flounder

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Interest in the culture of flatfishes has increased in recent years due to the high consumer demand for these fishes. The southern flounder, Paralichthys lethostigma, is the focus of important commercial and recreational fishes in the south Atlantic and Gulf of Mexico. However, culture requirements of this species are poorly understood.

In 1996, two larval rearing studies were conducted with the southern flounder to examine the effects three larval diets and high and low light conditions have on survival, growth and pigmentation. At 24ºC, five-day old larvae (1.98 ± 0.1 mm TL) initiated feeding on the rotifer Brachionus plicatilis and completed metamorphosis by day 30. In treatment 1 larvae (6 days post-hatch (dph) were fed rotifers (10/ml) days 6-15 and Artemia nauplii (3/ml) day 7 through metamorphosis. The second treatment was fed rotifers from 6 dph through metamorphosis and Artemia 7 dph through metamorphosis, while treatment 3 was fed using the same protocol as treatment 1 plus a commercially prepared larval diet.

Metamorphosis began on day 23 (8.2 ± 0.6 mm TL) and was completed one week later in both studies. No differences in size (11.4 ± 1.2 mm TL), survival (37.5 ± 15.6) or percent natural pigmentation (30.5 ± 12.7) were detected. Treatment 1 was also evaluated in low light (42-387 lx) and high light (801-1820 lx) conditions. Similarly, no differences were detected among treatments reared under low light conditions. No significant change in pigmentation patterns was detected 1 week after metamorphosis.

Current Status of Atlantic Halibut Culture in the Maritimes

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Research on Atlantic halibut has been ongoing at the St. Andrews Biological Station since 1990. Several studies, using wild-caught juveniles, have been undertaken to determine the suitability of the Bay of Fundy area for outgrowing. However, the majority of research effort has focused on solving problems related to the mass production of juveniles. One major problem has been poor survival in the yolk-sac stage which, in this species, is up to 45 days long. This presentation describes methods used to improve survival and larval quality using salinity manipulation. In 1996, the first commercial production of Atlantic halibut juveniles was achieved through a DFO/industry partnership. A description of the commercial rearing techniques used and the problems encountered is presented.

Reproduction of American Plaice, *Hippoglossoides platessoides*, and Turbot, *Reinhardtius hippoglossoides*, in Newfoundland Waters

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Maximizing reproductive output within the somatic constraints imposed by relatively small body cavity in plaice and turbot is likely accomplished through a batch-spawning strategy. Evidence of production and release of distinct batches of oocytes is given for wild American plaice and a similar strategy is suspected for turbot based on initial investigation. Increase in white muscle moisture during the spawning season, observed in American plaice, may indicate the need to draw on protein reserves to push oocytes through vitellogenesis to ovulation. The increase in "jellied" turbot in commercial catches also corresponds to the summer breeding season and represents protein utilization probably routed into production of oocyte batches. There is some evidence that plaice may forego reproductive development in the face of energetic constraints.

Investigation into the Causes of Early Larval Mortality in Cultured Summer Flounder

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Culture of larval marine fishes has long been a problem. In order to increase hatchery production, for either commercial aquaculture or stock enhancement, the cause of mortality in the early larval period, especially during the critical period of first feeding, must be overcome. This experiment was designed to investigate the first-time feeding period in summer flounder (*Paralichthys dentatus*), specifically to determine to what degree mortality is caused by the failure of larvae to initiate feeding. Larvae from individual male x female crosses were cultured in replicate bowls. Mortality rates of larvae in bowls that received food (live rotifers) were compared to those in bowls that did not. In addition, larvae were subsampled from each bowl each day to determine percentage of larvae with food in the gut. Larvae from one cross were also subjected to an experiment in which some of the replicate bowls contained "green water" (algae added) and some contained "clear water" (no algae) (All other experiments were conducted using clear water). Mortality was not due to inability of the larvae to initiate feeding. Large-scale mortality in fed replicates was observed often prior to mortality of unfed control fish and daily subsamples of fed larvae indicated that a high percentage had food in the gut regardless of mortality rate. Inter-replicate variability (*i.e.*, among fed bowls from a single parent cross) was extremely high. Results of the green-water vs. clear-water experiment demonstrated significant differences in growth, but not survival. Our results, especially inter-replicate variability, suggest that something in the rearing environment (differing from bowl to bowl) was responsible for the early larval mortality.

Progress in Controlled Maturation and Spawning of Summer Flounder (*Paralichthys dentatus*) Broodstock

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Wild-caught, adult summer flounder (*P. dentatus*) (n=60, avg. wt.=740 g; range=264-1540 g), collected during September and October 1994 off New Haven, Connecticut and Tuckerton, New Jersey, were transported to Vero Beach, Florida in March 1995 and held in six, 2.6-m^3 fiberglass tanks through November 1995 under three different photothermal regimes: (1) indoor, artificial photothermal conditions simulating natural habitat regimes (natural regime); (2) conditions as in (1) but with seasonal temperatures changes advanced by one month (accelerated regime); and (3) outdoor, ambient conditions in Vero Beach, Florida (ambient regime). Under the natural and accelerated regimes, day length and temperature were held constant after declining to autumnal levels of 10 hr and 17ºC, respectively. Under all treatments, onset of vitellogenesis in females (monitored by biopsy) was associated with declining day length and temperature conditions, with ovarian maturation beginning in the accelerated treatment group, then progressing to the natural and ambient groups.

From 20 September to 28 November 1995, twenty-four hormone-induced spawning trials were conducted with females from the accelerated and natural regimes using a single intramuscular implantation of a cholesterol-cellulose pellet containing LHRH-a (100 mg/kg body wt). Females with initial mean oocyte diameters of 258-456 µm spawned voluntarily 2.5-5.5 days post-implantation, while no response was obtained from females with smaller mean oocyte diameters ranging from 151-231 µm. Two females were spawned twice by LHRH-a pellet implantation. Infrequent, natural spawning without hormone induction was also obtained. Females released from 22.7-396.9 x 10^3 eggs on the first day of spawning, with fertilization rates ranging from 0-93.4% and hatching rates ranging from 0-81.1%. Variable fertilization rates were attributable to inconsistent performance by males.

The results demonstrate that LHRH-a-induced and natural spawning of photothermally-conditioned fish are promising techniques to help meet increasing year-round demands for summer flounder seedstock.

Production of Summer Flounder at Great Bay Aquafarms

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No Abstract available

Abstracts

Poster Presentations

Maturity Stages of American Plaice in the Gulf of St. Lawrence

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American plaice in the Gulf of St. Lawrence, and throughout much of their range, migrate in late autumn to deeper water. The overwintering period that follows involves a cessation of feeding and rapid development of gonads, terminated by an early springtime migration to spawning areas. Groundfish surveys conducted in summer months frequently report difficulty in identifying the maturing reproductive stages, particularly in identifying plaice destined to spawn the following spring. This poster outlines work that we have undertaken to classify maturity stages of plaice throughout the annual cycle, based on morphological and histological observations.

Can Scales be Used to Sex Winter Flounder (*Pleuronectes americanus*)?

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For finfish species such as winter flounder, which exhibit little if any sexual dimorphism, the ability to sex individuals without sacrificing them is problematic. However, the literature suggests that the sexes can be differentiated by the character of the scales on the blind side (*i.e*., ctenoid for males and cycloid for females). To determine the reliability of this dimorphism, a total of 730 fish were collected from both inshore and offshore locations. Prior to examining the gonads, the blind side of each fish was palpated noting either roughness (males) or smoothness (females). In addition, scales were sampled from 672 fish to ascertain the influence of observer error. Overall only 75% of the identifications were correct, with an error rate of 45% for males and 6% for females. Observer error accounted for 20% of the misidentifications. The effects of utilizing this methodology on estimates of various populations parameters (*e.g*., sex ratios, size structure) will be discussed.

Witch Flounder (*Glyptocephalus cynoglossus*) Aquaculture

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Although witch flounder (*Glyptocephalus cynoglossus*) consistently fetch the highest prices of any groundfish species landed in New England (larges have averaged \$3.80-\$5.15/lb thus far in 1996), few, if any, attempts at aquaculture have been made. A lengthy larval period, predicted fragility, and unpredictable timing and location of spawning have been the primary deterrents.

Witch flounder spawn from April through September in the Gulf of Maine. During June and July adult fish were stripped at sea. Milt from several males was used to fertilize the eggs of each ripe and running female. Incubation proceeded in 40 kriesels with stocking densities of approximately 250 eggs per liter and an average water temperature of 7.5ºC. Mean egg diameter was 1.2 mm. Water was changed twice daily through the germ ring stage (due to significant excretion of ammonia during the blastodisc to germ ring transition) and daily thereafter. Fifty percent hatching was reached at an average of 115 degree days. Mean length at hatching was 4.92 mm. At 75% hatching, marine microalgae (*Isochrysis galbana* Tahitian strain) was added to kreisels at a density of 200,000 cells per ml.

Following yolk-sac absorption, larvae were transferred to 200 flow-through tanks. Microalgae was added twice daily at the above density. Mean length at yolk-sac absorption was 6.34 mm. First-feeding larvae were fed a combination of wild zooplankton and cultured rotifers (*Brachionus plicatilius*) from 48-200*i* at a density of 3000 prey per liter. Water temperature averaged 13ºC. Photoperiod was maintained at 17L:7D. Weekly mean increases in length were 19.8%, 11.3% and 29.2% for the three weeks post yolk-sac absorption, respectively. Higher temperatures caused by a problem with a water cooling system may have been responsible for the decline in growth rate during the second week. Witch flounder larvae survived temperatures from 5-19.5ºC, but appeared to grow optimally between 8-12ºC.

Witch flounder have thus far been raised to a maximum length of 47 mm (at this length metamorphosis has not yet occurred) and have been weaned onto a commercial dry diet.

Early attempts at experimentation were unsuccessful due to mechanical problems but future research will examine the following: effect of size and quality of live diets on growth and feeding of first-feeding larvae; effect of temperature on larval growth and mortality; effect of photoperiod on larval feeding and growth; optimal techniques for the collection and maintenance of adult broodstock.

Given current hatching success and larval hardiness, the potential for witch flounder aquaculture on a commercial scale may be quite high.

Pleurocidin -- An Antimicrobial Peptide from the Skin Secretions of Winter Flounder*

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Antimicrobial peptides are found in both myeloid cells and mucosal tissue of a wide variety of species. These peptides are predicted to function as a first-line host defense mechanism against pathogenic organisms. We report the characterization of a novel 25-residue linear antimicrobial peptide, pleurocidin, found in the skin mucous secretions of the winter flounder (*Pleuronectes americanus*).This peptide was purified to homogeneity through multiple chromatographic methods. This purified peptide exhibited antimicrobial activity against *E. coli* in a bacterial cell-lysis plate assay. Mass spectrometry, amino acid sequence analysis, and solid-phase synthesis of the peptide were subsequently performed. Pleurocidin is predicted to assume an amphipathic alpha-helical comformation similar to many other linear antimicrobial peptides. Although there is insignificant homology between pleurocidin and the cecropin, magainin, and defensin families of antimicrobial peptides, there exists a high degree of homology between this peptide and two antimicrobial peptides, ceretotoxin from insects and dermaseptin from amphibians. The Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of pleurocidin have been determined against *E. coli*, *Aeromonas salmonicida, Cytophaga aquatilis, Leucothrix mucor, and Pasteurella haemolytica*. This peptide is shown to exert broad spectrum antibacterial activity. Pleurocidin represents the first antimicrobial peptide found in teleost fish and may play an important role in their host defense.

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Effect of Density on the Growth of Summer Flounder, *Paralichthys dentatus*

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Since stocking density can affect growth and survival of cultured finfish, the commercial success of any aquaculture venture depends, in large part, on knowing what the optimal stocking density is. As a rule, increasing density in a tank beyond some upper limit tends to reduce growth and/or survival through mechanisms such as competition for space, increased metabolite production, and cannibalism. This has been shown in several flatfish species (*e.g.*, turbot, *Scophthalmus maximus*; Atlantic halibut, *Hippoglossus hippoglossus*; and Japanese flounder, *Paralichthys olivaceus*). Optimal stocking density is unknown, however, for summer flounder which has become an important commercially cultivated fish in the northeastern United States.

Three different density treatments were employed to determine the optimal stocking density for juvenile summer flounder. Density was measured as percentage coverage of the substrate. For example, in a 100% density treatment, the surface area of the fish equaled the surface area available on the bottom of the tank. Stocking density treatments of 100%, 150% and 200% were used. Fish surface was estimated by tracing live specimens from several different age classes on a 1 cm x 1 cm grid and counting the number of cm^2 grids within each outline. Total length was also measured for each specimen. Since a least square linear regression of length on area gave an $R^2 > 0.9$, it was possible to use total fish length to estimate total fish surface area.

Seven-to nine-month-old summer flounder were stocked into shallow, black plastic 5.5 liter aquaria at densities of 100% (n=8), 150% (n=12), and 200% (n=16). There were three replicates of each treatment. Treatments were connected to a closed-water recirculating system with a water temperature of 18ºC. The flounder were fed Moore-Clark™ formula feed twice daily to satiation. Random samples of fish were measured and weighed every two weeks. Densities were adjusted by increasing tank size when necessary. Data were analyzed using one-way analysis of variance.

Overall, the flounder grew in weight and length over the course of the experiment in all treatments. There were no significant differences in mean lengths and weights ($p > 0.05$) during the 5-week experiment except during the second week. Although significant differences ($p < 0.01$) were seen in length (100%=150%>200%), they were not seen later in the experiment, and likely were due to a small sample size. Towards the end of the experiment, a decrease in size was seen in the 100% and 150% treatments. Since the decrease was seen in both length and weight, this anomaly was probably a result of small sample size. The results of this study suggest that the highest density treatment, 200%, did not have any negative effects on the growth of summer flounder. The optimal stocking density is still unknown.

Cloning and Sequencing of Winter Flounder Digestive Enzyme Genes

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The ontogeny of digestive enzyme function in flatfish has been studied using histochemistry and biochemistry but very little is known at the molecular biological level. Since larval winter flounder are very difficult to wean onto artificial diets at an early age, it is of great interest to know the timing of digesting enzyme expression during development. Molecular techniques such as reverse transcription-PCR (RT-PCR) provide a very sensitive measure of gene expression from minute quantities of tissue or even single cells. In order to acquire the information necessary to establish RT-PCR assays for larval winter flounder, we are isolating cDNA clones for various digestive enzymes.

Three cDNA libraries have been constructed from intestine, pyloric caeca and pancreas in the ëZAP vector (Stratagene). Portions of the trypsin, elastase, aminopeptidase and amylase genes have been prepared by PCR amplification using primers based on conserved sequence motifs, and their identity confirmed by sequencing. The PCR products were cloned into the plasmid vector pCR2.1 (Invitrogen) and used as radioactively-labeled probes to screen the ëZAP libraries. This has resulted in the isolation of several cDNA clones for elastase, trypsin, and aminopeptidase and represent the first cDNAs for winter flounder digestive enzymes, as well as the first elastase and aminopeptidase cDNAs for any fish. Sequence analysis of these clones will be presented as well as Southern hybridization analysis of gene copy number. Screening for amylase cDNAs is in progress.

From this sequence information, primers can now be designed that can be used to specifically amplify coding sequences of digestive enzymes of interest from whole larvae at different developmental states by RT-PCR. Probes will also be developed that can be used for *in situ* electron microscopy to identify which cells or portions of the larval gut are producing specific enzymes. These will be invaluable tools for estimating when larval digestive tracts are competent to assimilate exogenous food, and investigating genetic, physical or environmental parameters affecting digestive function.

Juvenile Winter Flounder Injected Subcutaneously with Elastomer: Movements and Mortality Estimates in Two Selected Coastal Salt Ponds

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Limited movement of young-of-the-year (YOY) winter flounder (*Pleuronectes americanus*) was found from May through October of 1996. During 1996, YOY winter flounder were tagged subcutaneously with visible impact fluorescent elastomer (VIE) and released at one station in Point Judith Pond and the Narrow River, in conjunction with the ongoing juvenile finfish survey being conducted in Rhode Island coastal ponds. Recaptures occurred during all months. These were 906 and 917 YOY winter flounder tagged in Point Judith Pond and the Narrow River, respectively. Of the recaptured fish, 100 percent were within the tagging station. None were found at any of the regular juvenile survey stations in Point Judith Pond or the Narrow River, suggesting limited movement. Survival estimates will be calculated when the data set is completed.

Rearing Juvenile Summer Flounder, *Paralichthys dentatus*, in Different Temperature and Salinity Combinations to Enhance Growth for Aquaculture

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Juvenile flounder were reared on a commercial diet under different temperature and salinity regimes to determine best growth conditions for aquaculture, and whether the fish could be reared at low salinities. Temperatures used were 16, 20, and 24ºC; salinities 10, 20 and 30 ppt. Significant differences in growth occurred. Two-way ANOVA showed significant effects of temperature with high temperatures providing the best growth. There were no significant differences between salinities, nor was there a significant interaction between temperature and salinity on growth. Fish at 24ºC increased weight by 320% and length by 53%; those at 20ºC increased weight by 175% and length 34% and at 10ºC increased weight by 130% and length 25%. High levels of nitrogen (nitrite, nitrate, ammonia) occurred in a preliminary experiment which produced some interesting results.

The conclusions are that temperature has a strong effect on growth but salinity does not; thus, fish can be reared at 10 ppt with no apparent loss in growth potential.

Ecology of Sheepscot Bay Winter Flounder

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The winter flounder population in Sheepscot Bay has been studied, from 1988 through 1994, using otter trawl, fyke net and beach-seine surveys at various locations throughout the Bay. We have documented, through the examination of otolith daily growth rings, that winter flounder spawn in the region primarily during June but some fish spawned as late as July. The larvae settle out of the water column and occur as juveniles in intertidal fyke net samples as early as June but may occur in low numbers as late as December. Over the four years of sampling, from 1990 through 1994, a consistent pattern of occurrence was observed. A rapid rise in September was followed by a peak in abundance, usually in October. There was an equally precipitous decline in the catch in November dropping to very low numbers in the December samples. The mean size of fish ranged from 4.0 to 8.0 cm at the peak abundance in September and October.

Trawl catches near Sequin Island, at a depth of 19 fathoms, showed a very strong correlation between winter flounder occurrence and temperature. The flounder catch steadily increased from a low in March to a peak in September and October as the temperature ranged from 1.5 ºC in March to 10.0 ºC in September. Juvenile fish dominated the catch all year with the mean size of 17 cm and a range from 7 to 38 cm in total length. The peaks in abundance at both the intertidal station and the Sequin tow occurred during the autumn, suggesting a strongly temperature-driven system with flounder abundance reaching its low during the late winter and early spring when water temperatures are at their nadir.

As the groundfish stocks continue to decline the question of stock enhancement has arisen. In Maine, a legislatively-created Groundfish Hatchery Study Commission was established to investigate the feasibility of enhancement as a management tool, for example. The basic biological question remains, however, can hatchery-reared groundfish be utilized to augment wild stocks of fish? If enhancement can be demonstrated successfully then the question is one of scale and economics. It is our suggestion that the winter flounder population in Sheepscot Bay may be a model population for researching this question. To this end winter flounder larvae were obtained from Dr. Hunt Howell at the University of New Hampshire this spring. These larvae were reared on algae, rotifers, Artemia, and wild plankton. They metamorphosed after 35 days and continued to grow with very low mortality rates until August. Less than optimal rearing facilities resulted in an increased oxygen demand when we were trying to wean the fish to artificial food and we lost our fish. Nevertheless, Dr. Howell has maintained a culture of juveniles and tentative arrangements were made to tag these fish with visual implant tags for preliminary parallel release and recapture experiments in the Sheepscot and Great Salt Bays. Hatchery rearing of winter flounder will be attempted next year at the Department of Marine Resources Laboratory for more extensive field experimentation.

Light Intensity and Salinity Effects on Eggs and Yolk-sac Larvae of the Summer Flounder *Paralichthys dentatus*

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The effects of light intensity and salinity on eggs and yolk-sac larvae of summer flounder, *P. dentatus*, were examined under controlled laboratory conditions. Fertilized eggs (early gastrula stage), obtained by induced spawning of captive broodstock, were stocked (53 eggs/L) into fourty-eight 5-L, translucent containers under light intensities of 0 (constant dark), 500, 1000, and 2,000 lux and at salinities of 26, 31 and 36 g/L. Temperature was 19ºC and photoperiod was 12 L:12 D. Light intensity and salinity produced small, but significant ($P < 0.05$) effects on larval notochord lengths at the 97% yolk-sac absorbed stage (114-131 hr post-fertilization =hpf), at first feeding (129.5-135 hpf), and at yolk exhaustion (153.5-159 hpf), which were generally maximal at low light intensity (500 lux) and high salinity (36 g/L) and minimal at high intensity (2,000 lux) and low salinity (26 g/L). Yolk utilization efficiency declined ($P < 0.01$) with increasing light intensity, presumably due to light-induced activity. Assuming that largest larvae have maximum foraging and escape abilities, a low light intensity (500 lux) and a high salinity of 36 g/L were optimal for eggs and yolk-sac larvae, conditions consistent with shelf waters where eggs and early larvae of summer flounder prevail in nature. High survival (85.1%) to yolk exhaustion under all treatment conditions reflects an adaptability for inshore movement during the pelagic larval phase.